09/07/16

Transformation of DH5 alpha with the "InterLab transformation protocol":

DNA constructions were dried, so we resuspended them into 100µL nuclease-free water. We took 5µL of the product for 25µL of competent cells. All the experiment will be in ice to keep the competence of the cells.

Heat shock:

- 20'' in ice
- 45s at 42°C
- 2'' in ice

Recovery: 1mL of LB, 1h at 37°C.

Preparation of LB Cam plates (30ug/mL).

12/07/16:

Transformation of Top 10 (see the protocol of heat shock transformation above).

We spread 100uL on LB cam plates.

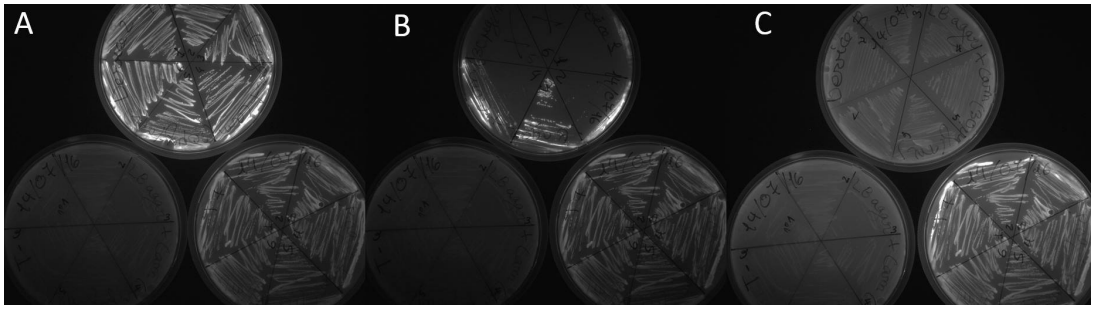
14/07/16:

Transformation seems have succeeded. We got few colonies on plates.

So we seed on plates to get more. We should do a PCR colony to check our plasmids. We calculated the length of PCR products by two known primers: VR and VF2

Expected PCR products length(VR/VF2):

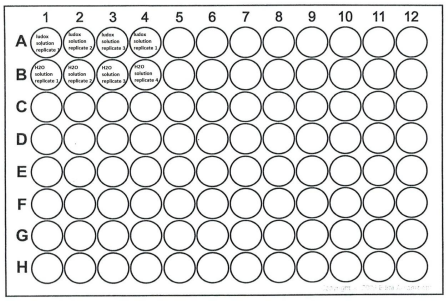
- Device 1, 2 and 3: 509 bp
- Negative control: 267 bp
- Positive Control: 1132 bp



Transformation of all samples from IGEM HQ. (a) Device 2 on the top, Negative control on the left, Positive control on the right; (b)Device 1 on the top, negative control on the left, Positive control on the right; (c) Device 3 on the top, Negative control on the left, Positive control on the right.

26/07/16:

*OD600 Reference point calibration
4 replicates of 100uL of Ludox (up)



4 replicates of -100uL of Ludox (down)

In OD600 mode:

	1	2	3	4
A	0.0885	0.076	0.0757	0.0765
B	0.0635	0.0661	0.0651	0.067

- Mean value of Ludox Abs(600): 0,079175
- Mean value of H2O Abs(600): 0,065425
- Corrected Abs(600) = Ludox Abs(600)-H2O Abs(600): 0,01375
- Corrected Abs***Correction Factor**=Reference Abs(600)=0.01475
- Correction Factor: **1,0272727**

01/08/16:

In OD600/GFP mode:

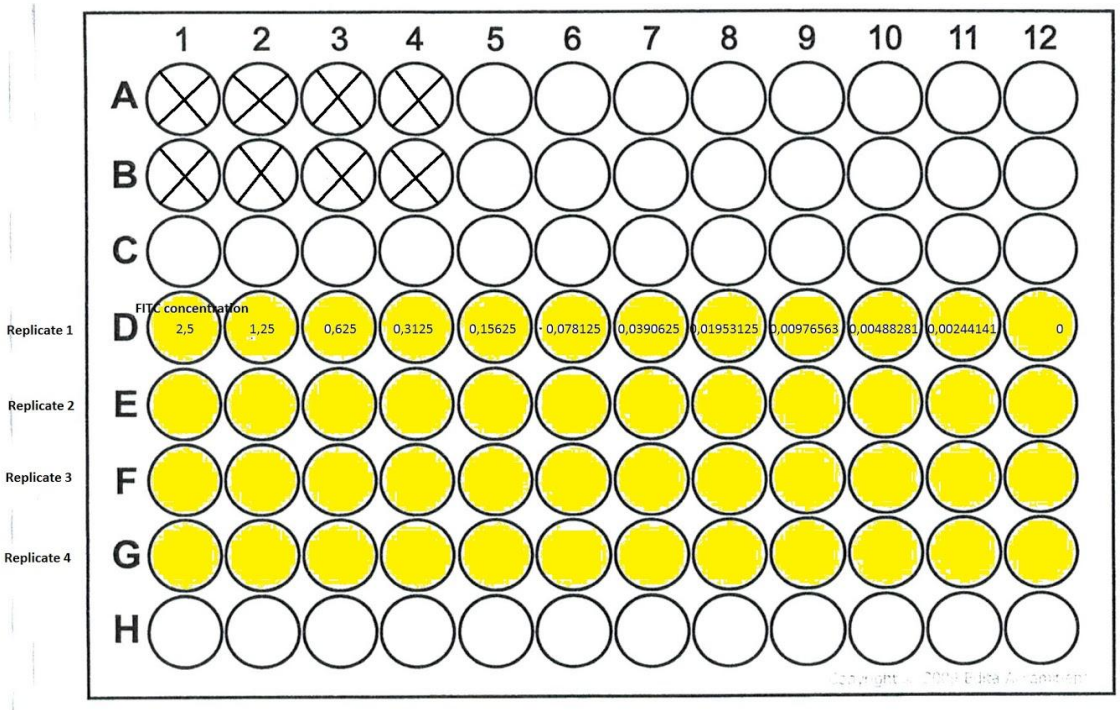
	1	2	3	4
A	SM1_1 1/1 0.0785	SM1_3 1/1 0.0795	SM1_5 1/1 0.0767	SM1_7 1/1 0.0788
B	SM1_2 1/1 0.069	SM1_4 1/1 0.0698	SM1_6 1/1 0.0648	SM1_8 1/1 0.0651

- Mean value of Ludox Abs(600): 0,078375
- Mean value of H2O Abs(600): 0,0671
- Corrected Abs(600) = Ludox Abs(600)-H2O Abs(600): 0,011275
- Corrected Abs***Correction Factor**=Reference Abs(600) = 0.01475
- Correction Factor: **1,30820399**

01/08/16:

Standard Curve in different relevant modes

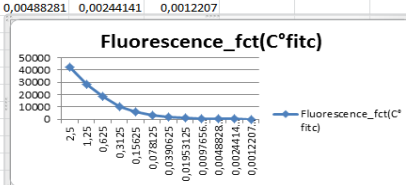
Standard curve Optimal Gain 100%



FLUORESCENCE

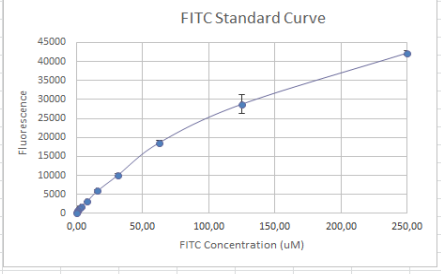
Replicates	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
	41924	28437	18180	10070	5568	3237	1809	1292	888	618	446	174
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
	41763	25327	18413	10331	6148	2971	1887	987	644	469	308	170
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
	42780	30795	18531	9502	5756	2976	1815	1277	780	475	369	173
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	42588	30544	19340	10353	6003	3186	1844	1200	840	588	469	184
Mean	1	2	3	4	5	6	7	8	9	10	11	12
	42263,75	28775,75	18616	10064	5868,75	3092,5	1838,75	1189	788	537,5	398	175,25
Ecart relatif	0,02406317	0,19002111	0,06231199	0,08455882	0,09882854	0,08601455	0,04242012	0,25651808	0,30964467	0,2772093	0,40452261	0,07988588

FITC equivalent concentration (uM)											
2,5	1,25	0,625	0,3125	0,15625	0,078125	0,0390625	0,01953125	0,00976563	0,00488281	0,00244141	0,0012207



- The optimal gain value calculated is **44**.
- This will be the one that I keep!
- But if we could redo the standard curve in different modes, I mean changing the orbitals (not the gain) in order to get a righter standard curve… It will be amazing but we'll have to redo the cell measurement!

replicate 3	42780	30795	18531	9502	5756	2976	1815	1277	780	475	369	173
replicate 4	42588	30544	19340	10353	6003	3186	1844	1200	840	588	469	184
Mean	42263,75	28775,75	18616	10064	5868,75	3092,5	1838,75	1189	788	537,5	398	175,25
SD	495,9277	2530,659	504,2175	396,1018	257,6605	138,9928	35,6125	140,5679	105,6788	76,65725	73,67949	6,075909



Optimal Gain=100%
Optimal Gain value=44

Pre-culture:

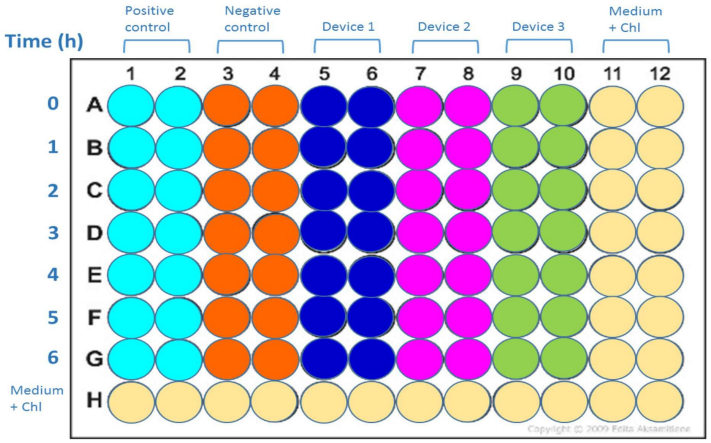
- 5mL LB
- 5uL Cam
- one colony from plate for T+, T-, devices 1, 2 and 3

13/08/16:

We measure the OD_{600} of each pre-culture, the table bellow calculates the volumes of LB pre-culture to add to TB volume to get an final volume of 10mL with an OD_{600}=0.02

1	target Abs600		0,02	
2	target volume (mL)		10	
3	sample	Abs600 re	volume o	volume of preloading media
4	positive control	1,501	0,137363	9,862637
5	negative control	0,496	0,443459	9,556541
6	device 1	1,463	0,141044	9,858956
7	device 2	1,06	0,197044	9,802956
8	device 3	1,473	0,140056	9,859944
9	media+chl	0,045	#DIV/0!	#DIV/0!
10	media+chl 1X	0,004		
11	media+chl 0,5X	0,004		
12				

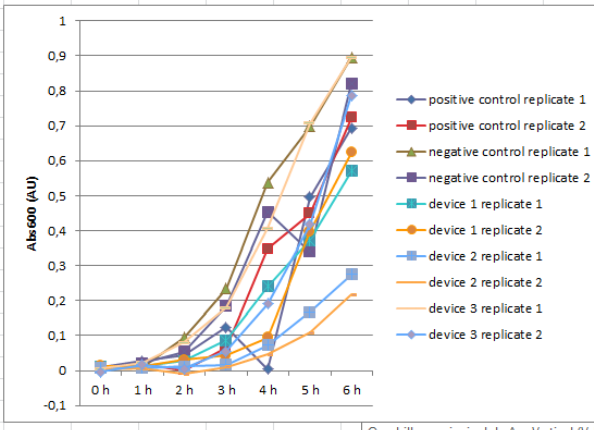
Then incubated them in 37°C and 2200rpm, taking samples each hour during six hours. Samples will be in ice then put in duplicates in 96well plate following the schema bellow.



We did it for Two media concentrations: 0,5 and 1 X.

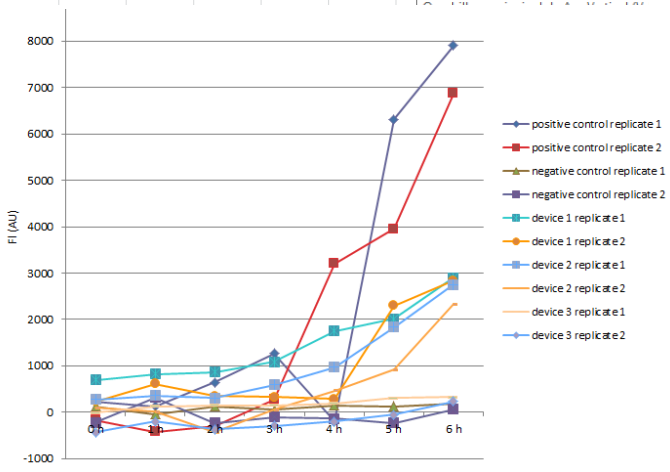
For 0,5:

replicate	replicate	replicate	replicate	replicate	replicate	enicol	
0,0874	0,0896	0,0852	0,0836	0,0839	0,0767	0,0793	0,0816
0,0891	0,0895	0,086	0,0841	0,0978	0,0924	0,0788	0,0802
0,1008	0,1034	0,0886	0,0722	0,1415	0,0845	0,0816	0,0796
0,1456	0,1126	0,0929	0,0879	0,2171	0,1217	0,0784	0,0786
0,2628	0,1517	0,1345	0,1146	0,3887	0,2251	0,0782	0,0783
0,3628	0,3821	0,2077	0,1624	0,6177	0,3974	0,0804	0,0817
0,5134	0,5535	0,2887	0,2451	0,7607	0,6772	0,0833	0,0792
					blank average		0,079943
					correction factor		1,316964



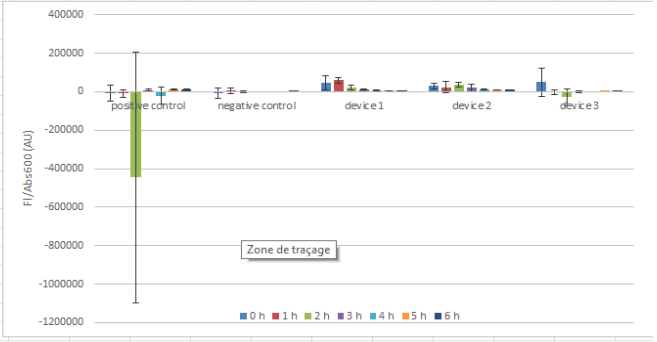
device 1		device 2		device 3	
replicate	replicate	replicate	replicate	replicate	replicate 2
0,009821	0,012718	0,006923	0,004816	0,005211	-0,00427
0,01206	0,012586	0,007977	0,005475	0,023517	0,016406
0,027468	0,030892	0,011401	-0,0102	0,081069	0,006002
0,086468	0,043008	0,017064	0,010479	0,180631	0,054993
0,240816	0,094502	0,07185	0,045642	0,406622	0,191167
0,372513	0,39793	0,168252	0,108593	0,708207	0,41808
0,570848	0,623658	0,274926	0,217506	0,896533	0,786566

device 1		device 2		device 3		media+chloramph	
replicate	replicate	replicate	replicate	replicate	replicate	enicol	
1322	860	900	717	617	190	592	599
1452	1243	983	632	753	423	614	625
1492	976	927	191	775	258	615	623
1716	957	1215	682	773	334	632	637
2378	908	1601	1087	821	428	629	625
2643	2921	2457	1544	927	577	640	626
3522	3457	3382	2951	954	859	633	678
					blank average		626,2857



device 1		device 2		device 3	
replicate	replicate	replicate	replicate	replicate	replicate 2
695,7143	233,7143	273,7143	90,71429	-9,28571	-436,286
825,7143	616,7143	356,7143	5,714286	126,7143	-203,286
865,7143	349,7143	300,7143	-435,286	148,7143	-368,286
1089,714	330,7143	588,7143	55,71429	146,7143	-292,286
1751,714	281,7143	974,7143	460,7143	194,7143	-198,286
2016,714	2294,714	1830,714	917,7143	300,7143	-49,2857
2895,714	2830,714	2755,714	2324,714	327,7143	232,7143

device 1		device 2		device 3	
replicate	replicate	replicate	replicate	replicate	replicate 2
70840,96	18376,49	39534,27	18834,75	-1781,8	102157,2
68469,29	48998,4	44717,62	1043,742	5388,149	-12391,2
31517,07	11320,46	26375,79	42687,35	1834,426	-61364,6
12602,5	7689,547	34500,18	5316,617	812,2318	-5314,99
7274,068	2981,053	13566	10094,04	478,8581	-1037,24
5413,813	5766,626	10880,81	8450,944	424,6136	-117,886
5072,658	4538,89	10023,49	10688,04	365,5352	295,861



SD

device 3	positive c	negative	device 1	device 2	device 3
50187,72	42562,17	28396,57	37097,98	14636,77	73496,01
-3501,54	19541,25	14275,77	13767,99	30882,1	12571,92
-29765,1	652075,1	3952,236	14281,15	11534,01	44688,49
-2251,38	4317,616	556,2863	3473,981	20635,89	4332,603
-279,191	43416,59	405,0766	3035,62	2455,046	1072,043
153,3638	2779,203	611,9782	249,4764	1718,178	383,6051
330,6981	1337,249	99,00927	377,4308	469,9124	49,26709

<u>Results for 0,5 X concentration aren't good too spare</u>
<u>Neither for 1 X concentration</u>

But we observed a greater fluorescence values in 1X but lower OD_{600} values meaning the bacteria seem to grow better on 0,5X concentration.
The Flu/OD600 ratio was highest in 1X.
We cannot compare the values difference of replicates because it the second values are missing for the 1X concentration.

We did not add the chemical compound needed in the TB media.

20/08/16

Preparation of Phosphate buffered saline 1X solution

- 8g NaCl for final concentration [137mM]
- 0.2 KCl [2.7mM]
- 1.44g Na_2HPO_4 [1mM]
- 0.24g KH_2PO_4 [1.8mM]

Add 800µL dH20 and mix. Then, adjust pH=7.4 (7.2 if necessary) with HCl.
When the pH is good, qs 1L. Sterilize by autoclaving.

22/10/16

Preparing the FITC stock solution

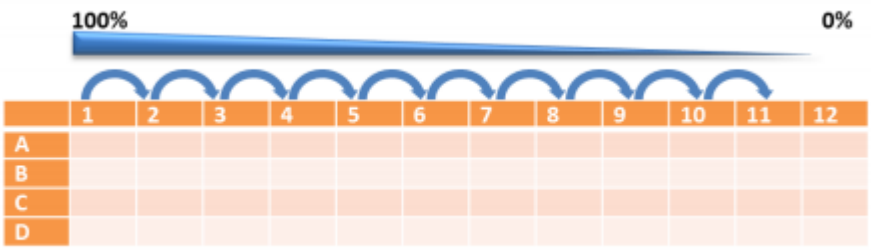
Spin down the FITC stock (FITC=194.7µg in the tube). Add 1mL of 1X PBS to have a stock solution of FITC at 500µM. Incubation at 42°C during four hours. After, I let the FITC in 42°C overnight because it is not dissolve totaly.

23/10/16

Preparing the FITC 1X solution

In a new 1.5mL tube, put 5ooµL FITC 2X with 500µL PBS 1X. We obtained a FITX 1X solution (250µM).

Preparing the serial dilution of the FITC 1X solution:



We did the serial dilution according the protocol from the interlab.

	250,00	125	62,5	31,25	15,625	7,8125	3,90625	1,953125	0,976563	0,488281	0,244141	0
replicate 1	39179	24758	15688	8776	4281	2402	1312	764	494	337	255	189
replicate 2	41426	27712	16128	9064	4744	2563	1382	808	497	349	265	198
replicate 3	41338	27653	16814	9116	4974	2600	1375	831	512	349	270	185
replicate 4	39655	27578	16594	9241	4753	2602	1426	810	509	350	266	193
Mean	40399,5	26925,25	16306	9049,25	4688	2541,75	1373,75	803,25	503	346,25	264	191,25
SD	1151,576	1445,874	501,5363	196,7289	291,4367	94,87667	46,94944	28,15878	8,831761	6,184658	6,377042	5,560276

We obtained these values.

25/08/16

Cell growth

→ Precultures of 2 colonies for each construction

Precultures of Device 1, 2, 3, negative control and the positive control in 5mL LB + 1µL Cam (30µg/mL), overnight (around 16 hours), 37°C, 220 rpm.

26/08/16

Measuring of OD_{600}

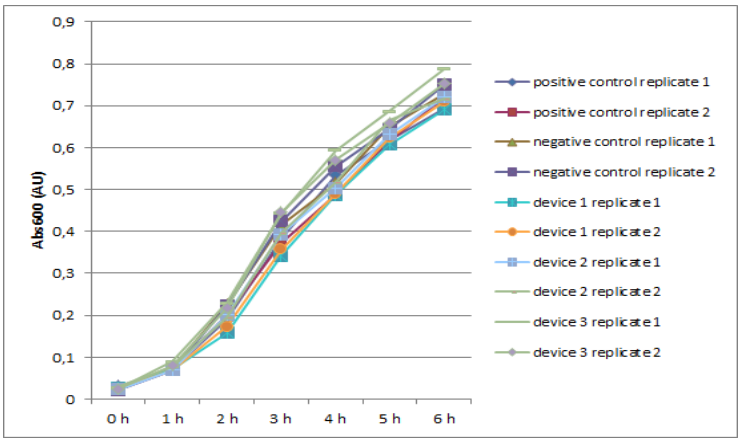
	Negative control	Positive control	Device 1	Device 2	Device 3
Replica N°1	0,5611	0,5711	0,5811	0,5811	0,6011
Replica N°2	0,5811	0,6211	0,6211	0,6811	0,5811

Volume to take off for a final OD600= 0.02

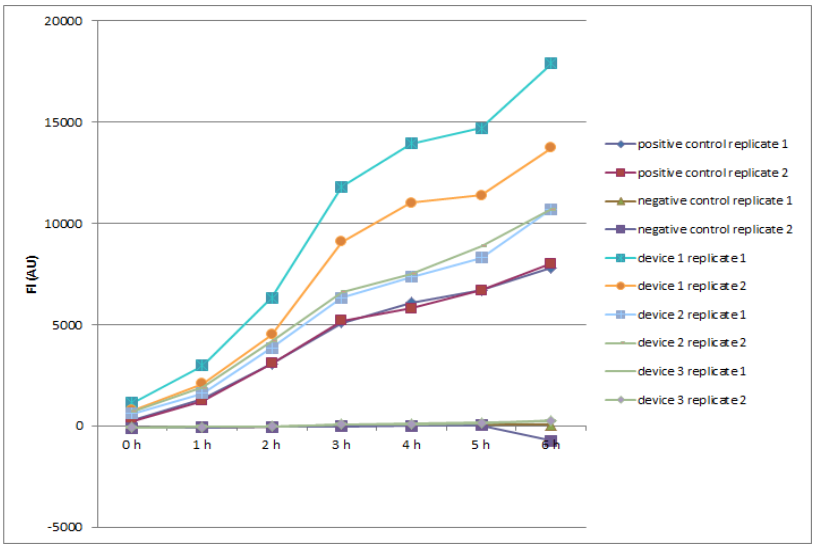
	Negative control	Positive control	Device 1	Device 2	Device 3
Replica N°1 (µL)	356	350	344	344	333
Replica N°2 (µL)	344	322	322	294	344

We add this volume in 10 ml 0.5x TB medium + Chloramphenicol (30µg/mL) in 50mL tube for the normal condition.

For all the samples (conditions with and without betaine and sorbitol), we took 250uL of each sample for the time t=0,
Incubate the cultures at 37°C and 220 rpm.
Each hour, we took 250uL until the time t=6. We kept the samples in ice until the last one.
Then, we put 200uL in the 96-well plate and we analyzed the OD and the fluorescence with a plate reader.



Absorbance in function of the time (condition without betaine and sorbitol)



Fluorescence intensity in function of the time (condition without betaine and sorbitol).

30/08/16

***Precultures of 2 colonies for each construction**

Precultures of Device 1, 2, 3, negative control and the positive control in 5mL LB + 1µL Cam (30µg/mL), overnight (around 16 hours), 37°C, 220 rpm.

01/09/16

***Preparation of 1x TB "Ready to use"**

Add 30mL of potassium phosphate solution in 300mL 1x TB "Step 1".

***Preparation of 0.5x TB "Ready to use"**

Add 60mL 1x TB with 60mL of steril water. Filtration with 0.22um.

***Preparation of 0.5x TB with chloramphenicol**

Add 72uL chloramphenicol in 120mL 0.5x TB.

- **OD600 measurement**

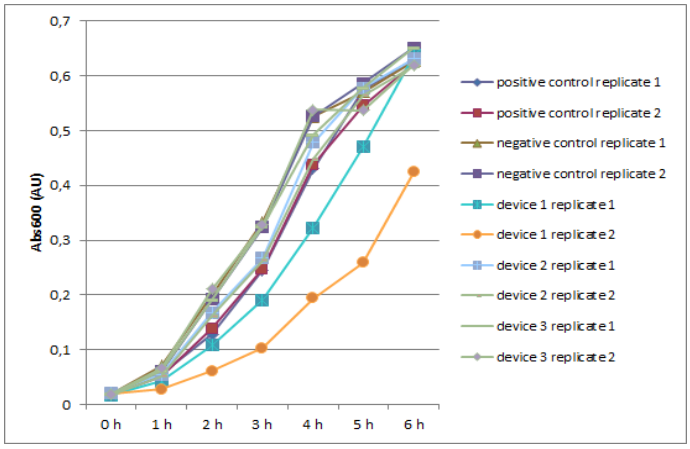
Replica n°1

target Abs600		0,02	
target volume (mL)		10	
sample	Abs600 re	volume of preload	volume of preloading media
positive control	0,6368	0,352174679	9,647825
negative control	0,6337	0,354107649	9,645892
device 1	0,6513	0,343406593	9,656593
device 2	0,6543	0,341646737	9,658353
device 3	0,673	0,331071015	9,668929
media+chl	0,0689		

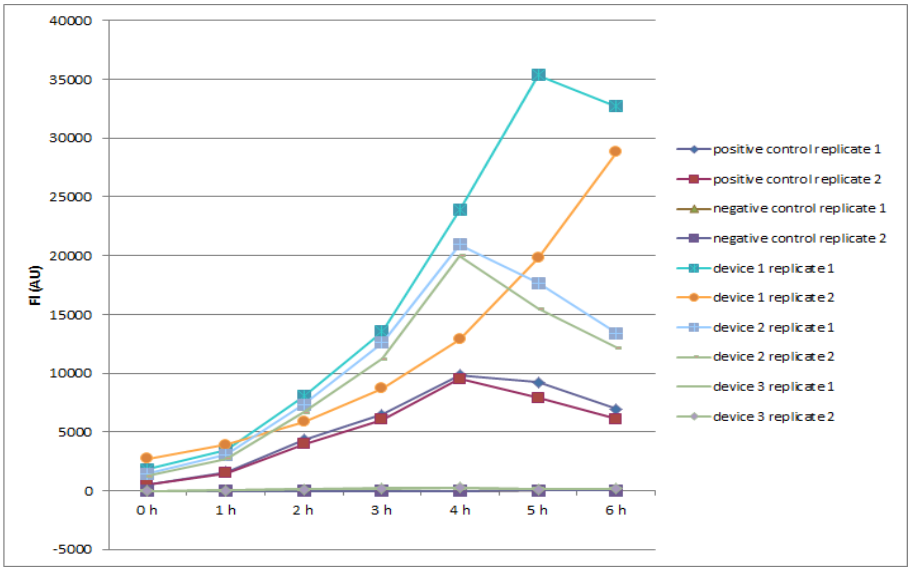
Replica N°2

target Abs600		0,02			
target volume (mL)		10			
sample	Abs600 re	volume o	volume of preloading media		
positive control	0,6925	0,320718	9,679282		
negative control	0,6491	0,344709	9,655291		
device 1	0,6914	0,321285	9,678715		
device 2	0,7542	0,291843	9,708157		
device 3	0,6546	0,341472	9,658528		
media+chl	0,0689				

For the condition with the betaine and the sorbitol, we did the next mix for a final volume = 100mL: 91.67mL 0.5x TB (with phosphate); 18.33 mL 5X sorbitol (2.5M); 916.67µL 100x betaine (500mM); 66µL cam (50mg/mL). Each hour, we took 250uL until the time t=6. We kept the samples in ice until the last one. Then, we put 200uL in the 96-well plate and we analyse the OD and the fluorescence with a plate reader.
We obtained the next result:



Absorbance in function of the time (condition with betaine and sorbitol)



Fluorescence intensity in function of the time (condition with betaine and sorbitol).